A Rapid Surface Intervention Process to Kill *Listeria innocua* on Catfish Using Cycles of Vacuum and Steam

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ABSTRACT: The vacuum/steam/vacuum surface intervention process was applied to catfish surface inoculated with *Listeria innocua*. Studies were performed to determine the optimum process conditions of steam temperature, steam time, and number of cycles. Cycling the treatment significantly improved the microbiological kill. At the optimum conditions of steam time of 0.05 to 0.10 s at 143 °C and with 4 cycles, bacterial kill in excess of 2-log cfu/ml (colony forming units/ml) was attained. The surface intervention process should ensure that catfish reaching the consumer have greatly reduced levels or are free of *Listeria* contamination.

Keywords: catfish, surface, intervention, process, *Listeria*

Introduction

DURING COMMERCIAL PROCESSING, AQUACULTURED CHANnel catfish are stunned, deheaded, eviscerated, skinned, filleted, chilled, washed, sorted, and packed (Kim and others 1994). These processing steps spread skin and gut microflora on work surfaces, processing equipment, and retail products such as fillets, steaks, nuggets, and whole fish. Several potential foodborne pathogens (*Salmonella* subsp., *Edwardsiella tarda* and *Listeria monocytogenes*) have been associated with catfish (Andrews and others 1977; Leung and others 1992; Meyer and Bullock 1973), catfish ponds (Leung and others 1992), and processing plants (Cotton and Marshall 1998). Although aquacultured channel catfish have never been incriminated in human outbreaks of enteric foodborne illness, these reports document the possibility of such an occurrence. The risk of cross-contaminating minimally processed and fresh products remains a concern both at home and in foodservice settings. There is a current demand by foodservice operators for fresh catfish products that are free from *L. monocytogenes* contamination. Presently there is no unit operation that can be used to deliver such a product.

Microbial control research has focused on catfish fillets using antimicrobial treatments such as organic acids and/or lactic cultures (Bal'a and Marshall 1998; Fernandes and others 1998; Ingham 1989; Kim and Hearnsberger 1994; Kim and others 1994; Kim and others 1995; Marshall and Kim 1996; Verhaegh and others 1996), modified atmospheric packaging (Silva and others 1993), organic salts (Williams and others 1995), and phosphates with organic salts (Kim and others 1995). Previously, we reported on the efficacy of short-term steam exposure on lowering bacterial counts on catfish skin (Bal'a and others 1999). The present study investigated the potential of a rapid vacuum/steam/vacuum (VSV) surface intervention process to inactivate *Listeria innocua*, which we used as a surrogate for *L. monocytogenes*. Pathogenic bacteria could not be used because all processing was done in a pilot plant restricted to food processing.

Morgan and others (1996a; 1996b) presented an excellent discussion of the theory behind the vacuum/steam/vacuum surface intervention process. In brief, steam is capable of killing pathogens, but air and water on the surface of the product act as insulation (Morgan and Carlson 1960; Perry and others 1984) although water is a good conductor of thermal energy, relative to steam, it acts as insulation. The time required to kill bacteria by steam alone by transferring the energy across the air/water barrier is sufficient to thermally damage the surface. This process called vacuum/steam/vacuum surface intervention employs a short exposure to vacuum to remove these insulating fluids. This is followed by a quick burst of condensing steam that rapidly transfers the energy directly to the bacteria. Then a second exposure to vacuum evaporatively cools the meat to prevent thermal damage. The process time is on the order of 1 to 2 s.

A prototype surface intervention processor was designed, fabricated, and patented (Morgan 1994; Morgan and others 1996a). Experiments utilizing this prototype showed the process reduces bacteria on fresh, raw chicken pieces by 2 to 3 logs. Further research led to improved prototypes. Near optimal processing conditions for chicken surface treatment were developed (Kozempel and others 2000a). These process conditions included a steam time of 0.05 s at 138 °C for 3 cycles. The surface intervention process also was applied to hot dogs (Kozempel and others 2000b). Optimum process conditions were determined for hot dog treatment compatible with current hot dog process line speed. Cycling the treatment significantly improved the microbiological kill. At the optimum conditions of steam time of 0.30 s at 138 °C cycled twice, bacterial kill in excess of 3 logs was attained. Pasteurization, frequently considered as bacteria kill above 5 logs, was achieved by increasing the number of cycles to 3.

Since catfish skin may be an important source of bacterial contamination during processing, efforts to achieve skin microflora reduction may prove worthwhile to enhance microbial safety and quality of catfish products. The present work was designed to obtain data on the impact of a rapid vacuum/steam/vacuum intervention process for inactivating *L. innocua* on catfish.

Materials and Methods

Catfish Preparation

Eviscerated and washed aquacultured channel catfish *(Ictalurus punctatus)* were obtained from a commercial processor and transported in ice to Mississippi State Univ. The fish were shipped in ice by overnight delivery to the Eastern Regional Research Center. The head and skin were left on. The average fish weight calculated from the total weight of fish per experiment divided by the number of fish, ranged from 270 g to 385 g.

The catfish preparation for preliminary investigation and for the actual study was the same except for harvest season and inoculation. In preliminary experiments to establish a feasible processing region, the fish were harvested in summer and processed as soon as received with no inoculation. They were analyzed for naturally occurring bacteria, APC (aerobic plate counts).

For optimization studies, the fish were harvested in winter, removed from the ice upon arrival, and stored in a refrigerator for 1 or 2 d before inoculation. The catfish were inoculated with *L. innocua* (SA3-VT, supplied by Pina Fratamico, Eastern Regional Research Center, Wyndmoor, Pa., U.S.A.). *L. innocua* was chosen because it is nonpathogenic and has similar or higher thermal resistance than *L. monocytogenes* (Ryser and Marth 1999).

To prepare an inoculum of *L. innocua,* a loopful of culture was removed from a refrigerated slant and added to 100 ml brain-heart infusion broth (BHI; Difco Laboratories, Detroit, Mich., U.S.A.) supplemented with 3% glucose. Inoculated BHI was incubated overnight at 28 °C. The amount of inoculum, contact and drain times were chosen by trial and error to have sufficient bacteria attached on the catfish for study. The catfish were inoculated by dipping into a container with 10^6 cfu/ml *L. innocua* for 10 min. This procedure was previously found (unpublished data) to give high bacteria counts when inoculating chicken. Bacteria counts were considered sufficiently high to do statistical studies when the inoculated bacteria were greater than 10^4 cfu/ml. Upon removal, the catfish were allowed to drain for 30 min before experimentation. After 30 min the excess surface liquid had drained and dried.

VSV Surface Intervention Processor Mechanical Design

The surface intervention processor was designed to process chicken carcasses, specifically broilers. This research was an attempt to adapt the process to raw catfish. The performance requirements of a surface intervention processor are to accept raw food products such as catfish individually and to enclose them in a chamber within a rotor; to evacuate that chamber; to treat it in that closed chamber with steam; to cool it with vacuum; and finally to eject it into a clean environment. The simplest design, 1 chamber in 1 rotor, was designed and constructed. Figure 1 shows a schematic diagram of the processor and Figure 2 shows details of the product treatment section. A cylindrical chamber for a broiler carcass should be about 200 mm in dia and 240 mm deep. Such a chamber is provided by an 8-inch ball valve. The same cylindrical chamber (product valve) was used to treat individual catfish in this study. Obviously, a different size and shape product valve would be used in a machine designed specifically to treat catfish instead of chicken carcasses.

To admit vacuum or steam into the closed chamber, 2 opposed 200 mm holes were bored through the stator at right angles to both the axis of rotation of the ball and to the centerline of the open chamber. Two gas valves are close coupled to these 200 mm ports and consist of a flat disk rotating against an inlet header, which holds PEEK (polyetheretherketone) seals. Each disk contains 2 holes, which, when stopped at one of the ports in the inlet header, permits gas to flow into the treatment chamber. Multiple holes reduce the rotor angular movement necessary for valve action and increase the cross sectional area for gas flow. Each disk is programmed independently and moved by its own servomotor. The servos were 50-joule units, capable of high acceleration and deceleration.

In order to expose all exterior surfaces of the test sample to treatment, a screen was installed at the midpoint of the product valve to hold the sample. The steam generator was charged with deionized water and the water boiled for 30 min to remove air. The vacuum receiver was adjusted to 70 mbar and its condenser coil was cooled to 4 °C.

VSV Surface Intervention Processor Operation

Each catfish was manually inserted into the treatment chamber of the surface intervention processor. The ball valve was rotated 90 degrees, with a servo, to seal the chamber from the outside atmosphere. Operation of the ball valve was computer controlled. The platter valves rotated to expose the sample to vacuum, then steam, and then vacuum again. With multiple cycles, the sequence of vacuum, then steam, was repeated multiple times. Process variables were steam temperature, steam time, and number of cycles. After treatment, the ball valve rotated back 90 degrees (opened) to expose the sample to atmosphere. The catfish sample was aseptically removed manually after treatment.

Microbiology Testing

To determine *L. innocua* counts after processing in the intervention processor, the catfish were placed in sterile plastic bags with Butterfield buffer solution (Difco Laboratories, Detroit, Mich., U.S.A.), ratio of 1 catfish/400 ml of buffer, and manually rinsed for 60 shakes. The catfish rinses were appropriately diluted with sterile 1% peptone water and plated onto Tryptose Agar (Difco Laboratories, Detroit, Mich., U.S.A.) using a spiral plater (Model Autoplate 4000, Spiral Biotech, Bethesda, Md., U.S.A.). The plates were incubated at 37 °C for 1 d. Colonies were counted and expressed as cfu/

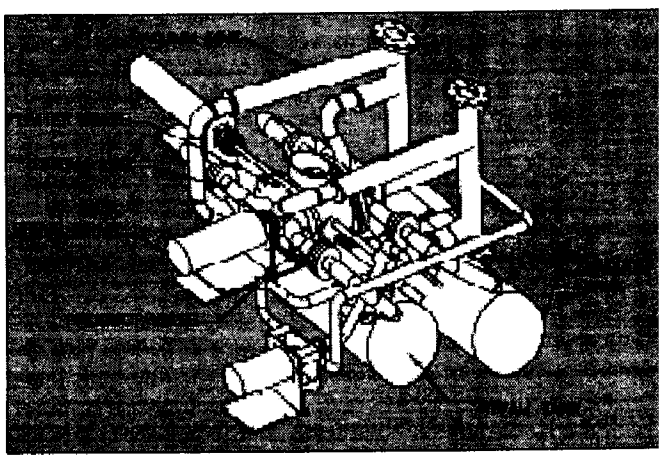

Figure 1—Schematic diagram of the prototype VSV surface intervention processor.

Table 1-2^3 Experimental design and analysis of variance for catfish, steam times of 0.05 and 0. 10 s

Experimental factors	Factor levels	
	–	+
A	138	143
B	0.05	0.10
C	1	2

A = steam temperature, °C
B = steam time
C = cycles
Initial vacuum time = 0.10 s
Intermediate or vacuum times between steam cycles = 0.50 s
Final vacuum time = 0.50 s

Experimental factors and interactions	*L. innocua* Mean, log cfu/ml	Mean square	F value
A	3.81	0.250	16.2***
B	3.87	0.126	8.2**
AB	3.71	0.0009	0.06
C	3.68	0.766	49.6***
AC	3.53	0.00015	0.01
BC	3.57	0.00195	0.13
ABC	3.36	0.00525	0.34
Error		0.0154	

Treatment samples consisted of 4 replicates.
Significant differences represented by; ** $p \leq 0.01$ *** $p \leq 0.001$
Control (8 replicates) = 4.54 Log cfu/ml (S.D. = 0.245)
Mean Square is the measure of variability attributed to the experimental factor or interaction.

Table 2-2^2 Experimental design and analysis of variance for catfish, steam times of 0.10 and 0. 15 s

Experimental factors	Factor levels	
	–	+
A	0.10	0.15
B	2	3

A = steam time
B = cyclesSteam temperature = 143 °C
Initial vacuum time = 0.10 s
Intermediate or vacuum times between steam cycles = 0.50 s
Final vacuum time = 0.50 s

Experimental factors and interactions	*L. innocua* Mean, log cfu/ml	Mean square	F value
A	3.54	0.0081	0.11
B	3.35	0.2304	3.01
AB	3.41	0.0484	0.63
Error		0.0767	

Treatment samples consisted of 4 replicates.
Control (8 replicates) = 4.76 Log cfu/ml (S.D. = 0.201)
Mean Square is the measure of variability attributed to the experimental factor or interaction.

ml. Because the catfish had very low bacterial contamination before inoculation, it was unnecessary to differentiate *L. innocua* from interfering background flora. The APC was less than 2 log cfu/ml. Non-inoculated controls had no detectable *Listeria* (detection limit 0.60 log cfu/ml).

To determine aerobic plate counts (APC), aliquots of serial dilutions were plated on Petrifilm™ Aerobic count plates (3M Microbiology Products, Minneapolis, Minn., U.S.A.) following the manufacturer's recommended procedures. The Petrifilms™ were manually counted after incubation at 37 °C for 48 h.

All dilutions were plated in duplicate. APC was used for preliminary studies with uninoculated catfish to establish feasible processing parameters. *L. innocua* counts were used for the 2^n factorial experimental designs.

Process Optimization

For optimization studies presented in Tables 1 through 3, 2^3 or 2^2 factorial experimental designs (Davies and others 1960) were used. Treatment samples consisted of 4 replicates. The data from the factorial designs were analyzed by ANOVA using the replicate within treatment terms as error terms. Control samples of inoculated catfish were taken to provide an independent estimate of the extent of bacteria kill. A null hypothesis (Volk 1958) was made on the difference between means (H_0; $mean_1 = mean_2$) to compare the mean bacteria counts at various process parameters.

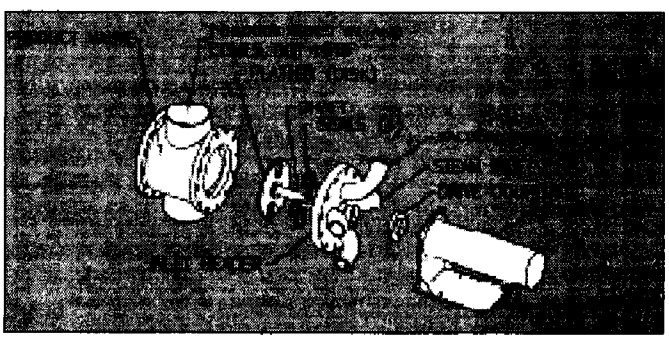

Figure 2—Details of the product treatment section of the prototype VSV surface intervention processor.

Results and Discussion

Previous research with chicken carcasses indicated that the optimum process parameters were a steam time of 0.10 s and steam temperature of 126 to 138 °C (Kozempel and others 2000a). Insufficient vacuum time following the steam period permitted visually detectable thermal damage to the flesh. A vacuum time of 0.10 s was too short, whereas 0.30 s appeared to be acceptable and 0.50 s assured an acceptable product. Because catfish is also susceptible to thermal damage, we chose 0.50 s for vacuum time for all studies to assure an acceptable product. Research with hot dogs indicated an optimum at steam time of 0.30 s at 138 °C cycled 3 times (Kozempel and others 2000b).

Preliminary Studies

Preliminary studies with uninoculated catfish were conducted to establish a feasible region for process variables to initiate optimization studies. Steam time was 0.10 s and the temperatures were 138, 143, and 149 °C. Initial vacuum time was 0.10 s and final vacuum time was 0.50 s. APC were determined for treated and untreated catfish. These catfish were harvested in summer. The mean APC of the control samples (n = 9) was 3.24 log cfu/ml (SD = 0.587). At 138 °C the APC mean count (n = 9) was 2.37 log cfu/ml (SD = 0.305). At 143 °C the mean APC count (n = 10) was 2.49 log cfu/ml (SD = 0.415). At 149 °C the mean count (n = 10) was 2.54 log cfu/ml (SD = 0.277). The log kills were 0.87, 0.75, and 0.70, respectively. All experiments were 1 cycle. There was a statistically significant difference between each treatment and the controls. There was no significant difference among the means of the treated samples. Previous work with atmospheric steam (100 °C) and longer steam contact times demonstrated similar log kills for APC, psychrotrophic counts, and

Table 3-2[3] Experimental design and analysis of variance for catfish, steam times of 0.10 and 0.15 s

Experimental Factors	Factor Levels	
	–	+
A	143	149
B	0.10	0.15
C	3	4

A = steam temperature, °C
B = steam time
C = cycles
Initial vacuum time = 0.10 s
Intermediate or vacuum times between steam cycles = 0.50 s
Final vacuum time = 0.50 s

Experimental factors	*L. innocua*		
and interactions	Mean, log cfu/ml	Mean square	F value
A	3.58	0.389	2.70
B	3.85	0.027	1.88
AB	3.17	0.330	2.29
C	3.33	1.115	7.99**
AC	3.05	0.00015	0.001
BC	3.10	0.1047	0.73
ABC	2.93	0.5434	3.77
Error		0.144	

Treatment samples consisted of 4 replicates.
Significant differences represented by; ** $p \leq 0.01$
Control (8 replicates) = 5.20 Log cfu/ml (S.D. = 0.059)
Mean Square is the measure of variability attributed to the experimental factor or interaction.

coliform counts on catfish treated for 30 to 120 s (Bal'a and others 1999).

Optimization

With the preliminary data as a starting point, a series of 2^3 or 2^2 factorial experimental designs was made to determine optimum process conditions for catfish inoculated with *L. innocua*, as listed in Tables 1 through 3. Because these catfish were harvested in winter they had extremely low levels of background flora. The APC was less than 2 log cfu/ml. For the process parameters, steam temperature, steam time, and number of cycles were set to 2 levels in each design. In Table 1, the first 2^3 factorial experimental design, all 3 main variables were statistically highly significant. The highest levels of the variables were better in each case, 143 °C, 0.10 s steam time, and 2 cycles. The mean of all steam-treated samples used in the design (n = 32) was 3.69 log cfu/ml *L. innocua* (SD = 0.222). There was a statistically significant difference between the mean counts (and kills) at the high and low levels of the variables. The bacteria kill at 143 °C, 0.94 log cfu/ml, was significantly greater than at 138 °C, 0.76 log cfu/ml. The bacteria kill at 0.10 s steam time, 0.91 log cfu/ml, was significantly greater than at 0.05 s, 0.79 log cfu/ml. The bacteria kill at 2 cycles, 1.01 log cfu/ml, was significantly greater than at 1 cycle, 0.70 log cfu/ml.

In the next experiment, a 2^2 design, Table 2, the high levels from Table 1 were used as the low levels. Number of cycles and steam time were not significant. The mean of all samples used in the design (treated) (n = 16) was 3.50 log cfu/ml *L. innocua* (SD = 0.284).

In the next design, Table 3, the number of cycles and steam temperature were increased. Number of cycles was statistically highly significant. Steam temperature and steam time were not significant. The mean bacteria kill at the high level of 4 cycles, 2.10 log cfu/ml, was significantly greater than at 3 cycles, 1.72 log cfu/ml. This level of inactivation was greater than that achieved for *L. monocytogenes* on catfish using 2% organic acids, where the best reduction in counts was 1 log (Bal'a and Marshall 1998).

Based on these results, the optimum processing conditions for catfish are steam temperature of 143 °C and steam time of 0.10 s. The steam time of 0.10 s was shown to be significantly different from 0.05 s in Table 1 where the temperature range was 138 to 143 °C but the design did not include 4 cycles. The steam time was double-checked at the optimum conditions. The process was run at 0.05 and 0.10 s steam time at 143 °C and 4 cycles. The mean of the control samples (n = 4) was 4.97 log cfu/ml *L. innocua* (SD = 0.335). The bacteria count (n = 4) at 0.05 s was 3.15 log cfu/ml *L. innocua* (SD = 0.229), which was significantly different (p 0.05) from the control. The bacteria count (n = 4) at 0.10 s was 2.87 log cfu/ml *L. innocua* (SD = 0.373), which also was significantly different (p 0.05) from the control. However, a null hypothesis comparing the results of 2 experimental treatments, 3.15 and 2.87 log cfu/ml *L. innocua,* indicated there was no statistically significant difference between the two. Hence, the optimum steam time is 0.05 to 0.10 s.

Figure 3 is a linear plot of all the kill data at 143 and 149 °C and at 0.05, 0.10, and 0.15 s steam time, which were shown to be not significantly different, compare with the number of cycles. It is readily apparent from the plot that the bacterial kill is strongly dependent (correlation coefficient r = 0.785) on the number of cycles. The correlation was: Kill = 0.26 + 0.45 (Cycles), where Kill is log cfu/ml *L. innocua* and Cycles is number of cycles. A correlation with number of cycles is understandable. Air and water form a film on the surface of the product. The film interferes with the rapid energy transfer from the steam to the catfish. Although the interfering surface layers are removed with the initial application of vacuum, the condensing steam itself continuously deposits an insulating water (condensate) layer during processing. Cycling between vacuum and steam effectively removes this redeposited water layer as soon as it forms and improves surface treatment.

It is imperative that the intervention treatment not thermally change the flesh. The mucous coating on the fish skin

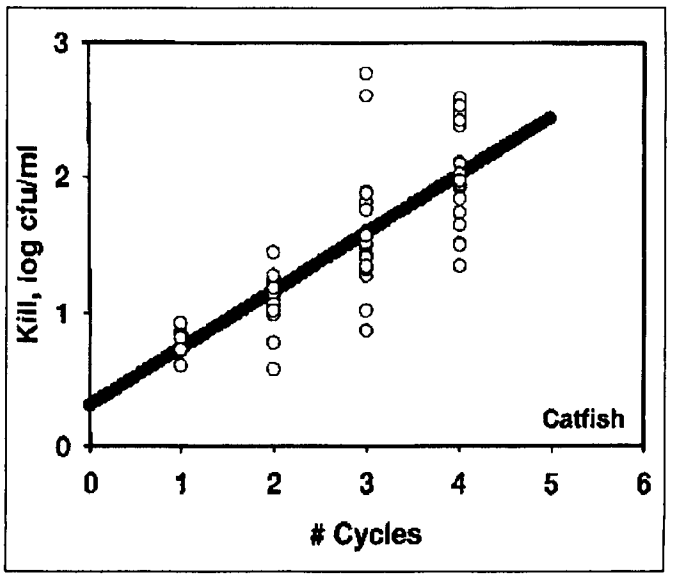

Figure 3—Effect of number of cycles on *Listeria innocua* present on the surface of catfish.

was affected. It visually turned to a dull gray color. However, the flesh beneath the skin was visually unaffected. Because the catfish are normally filleted the damage to the skin surface is not an issue. As previously mentioned this processor was designed to test chicken carcasses, not fish. The differences in the carcasses caused some problems. The tails and fins sometimes got wedged in the vacuum disk of the platter valve and were progressively "chewed up" mechanically with increasing cycles. Therefore, we chose 4 cycles as the optimum or limit. A unit designed for catfish should be capable of more cycles and probably increased microbiological destruction. A full series of experiments are planned to process catfish on-site with a field surface processor using the optimum conditions. The goals of these experiments will be the testing of the process as it would be applied commercially and the performance of sensory and microbiological evaluations.

The optimum processing conditions for catfish were 143 °C steam temperature and 0.05 to 0.10 s steam time per cycle. The data indicate the bacterial kill continues to increase with number of cycles, with 4 cycles being a practical and limiting number. There was no visible damage to the fish flesh.

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MS 20000715

This work was supported in part by a USDA-ARS Food Safety Cooperative Agreement #58-6202-0-001 and by the Mississippi Agricultural and Forestry Experiment Station under project MIS-0860. Mention of brand or firm names does not constitute an endorsement by the U.S. Dept. of Agriculture over others of a similar nature not mentioned.

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